Applications of GeoChip to examine functional microbial communities in metal contaminated environments

Joy D. Van Nostrand[1,2], Liyou Wu[1,2], Sanghoon Kang[1,2], Patricia Waldron[1,2], Yuting Liang[1,3], Ye Deng[1], Zhili He[1,2], Weimin Wu[4], Heidi Gough[5], Sue Carroll[6], Chris Schadt[2,6], Anthony Palumbo[2,6], Dave Watson[6], Craig Criddle[4], Phil Jardine[6], Brett Baldwin[7], Aaron Peacock[5], Phil Long[6], David Stahl[2,8], Terry C. Hazen[2,9], Jizhong Zhou[1,2]

[1]University of Oklahoma, Norman, OK; [2]VIMSS (Virtual Institute of Microbial Stress and Survival) http://vimss.lbl.gov/; [3]Tsinghua University, Beijing, China; [4]Stanford University, Stanford, CA; [5]University of Washington, Seattle, WA; [6]Oak Ridge National Laboratory, Oak Ridge, TN; [7]University of Tennessee, Knoxville, TN; [8]Pacific Northwest National Laboratory, Richland, WA; [9]Lawrence Berkeley National Laboratory, Berkeley, CA

ESPP2 Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

VIMSS Virtual Institute for Microbial Stress and Survival | MIT Massachusetts Institute of Technology | Missouri | University of Washington | Montana State University | Southern Illinois Carbondale | Oklahoma | Sandia National Laboratories | Oak Ridge National Laboratory | Berkeley | 75 | http://vimss.lbl.gov/

ABSTRACT

The GeoChip 2.0, a functional gene microarray, allows for the simultaneous detection of >10,000 genes involved in the geochemical cycling of C, N, and S, metal reduction and resistance, and organic contaminant degradation. The GeoChip has been used to examine microbial communities at contaminated sites in order to provide a better understanding of the changes stressors (e.g., metals) have on dynamic functional and structural microbial communities in natural systems. This understanding will ultimately provide a correlation with laboratory observations using these same stressor. Here, five examples of studies utilizing the GeoChip to examine microbial communities at metal contaminated sites are presented. The first three studies examined areas within the U.S. DOE's Field Research Center (FRC) in Oak Ridge, TN. (1) Microbial communities within a pilot-scale test system established for the biostimulation of U(VI) reduction in the subsurface by injection of ethanol were examined. The microarray data indicated that during the U(VI) reduction period, both FeRB and SRB populations reached their highest levels at Day 212, followed by a gradual decrease over the following 500 days. The U concentrations in the groundwater were significantly correlated with the total abundance of c-type cytochrome genes and with the total abundance of *dsrAB* (dissimilatory sulfite reductase) genes. Mantel test analysis of microarray and chemical data indicated a significant correlation between the U concentration and total c-cytochrome or *dsrAB* gene abundance. Changes in more than a dozen individual c-type cytochrome genes and more than 10 *dsrAB*-containing populations showed significant correlations to the changes in U concentration among different time points, indicating their importance in uranium reduction. (2) In a different study of the same system, the effects of dissolved oxygen (DO) and ethanol on the stability of the bioreduced area were examined. Canonical correspondence analysis (CCA) and Mantel test analysis revealed that ethanol and sulfide concentrations showed the greatest correlation to the functional community structure. Detrended correspondence analysis (DCA) showed a shift towards a different community structure after ethanol injections resumed compared to the periods of starvation and exposure to DO. Changes in the functional community structure were similar in both wells; however, the community in FW101-2 was more affected by DO than in FW102-3. This is most likely because FW101-2, located closest to the injection wells, had a greater increase in DO than FW102-3, located further from the injection well. Hierarchical clustering showed that cytochrome c genes grouped based on DO exposure, starvation, or ethanol addition, while dissimilatory sulfite reductase (*dsr*) genes grouped only by starvation or ethanol addition. However, when DO levels increased, the relative abundance of *dsr* genes decreased while cytochrome genes seemed unaffected. Overall, results indicated that ethanol was the main factor affecting community structure, although some changes could be attributed to DO. (3) In the third study from the FRC, analysis of groundwater monitoring wells along a contamination gradient revealed less overlap between wells with different levels of U and NO3 contamination. While diversity of nitrate-fixation genes decreased in NO3 -contaminated wells, the diversity of metal reduction and resistance genes did not correlate with metal concentrations. Signal intensity did, however, increase in heavily contaminated wells, indicating a larger percentage of organisms with metal- related genes. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. Individual principle component analyses (PCA) of the gene diversity and geochemistry of the wells separated them in similar ways. CCA indicated that pH was an important variable that correlated with gene diversity in the lowest-contamination well, while NO3 and U correlated with the most highly contaminated well. Overall, contaminant level appears to have significant effects on the functional gene diversity along the contaminant plume at the FRC. (4) We have also used GeoChip to examine a Uranium Mill Tailings Remedial Action (UMTRA) site (Rifle, CO). Two adjacent mini-galleries were driven to Fe-reducing and SO4-reducing conditions, respectively, in order to better understand the long-lived U (VI) loss and to constrain the relative impacts of SO4 and Fe (III) reduction. Cluster analysis results showed samples in the same locations grouped together, regardless of geochemistry. The *dsr* genes increased when conditions were driven to sulfate-reduction. DCAs of both the functional community structure and environmental conditions (Fe2+, H2S, DO, pH, conductivity, potential and Eh) showed background, Fe-reducing, and SO4-reducing samples clustered together, respectively. CCA of environmental parameters and functional genes indicated Fe2+ was the most significant geochemical variable for community structure. (5) Additionally, metal contaminated freshwater lake sediments (Lake DePue, IL) were analyzed to examine the link between functional genes and the environmental gradient of metal contamination. Based on non-metric multidimensional scaling (NMDS), the microbial communities were separated based on sampling regions. In addition, there were different groupings between samples with the highest levels of contamination and the lesser contaminated samples. These studies demonstrate the analytical power of the GeoChip in examining microbial communities and its ability to provide direct linkages between microbial genes/populations and ecosystem processes and functions. This is the first comprehensive microarray available for studying the functional and biogeochemical cycling potential of microbial communities.

METHODS

DNA was extracted from all samples using a freeze-grind method (Zhou et al., 1996). An aliquot of extracted DNA was amplified using a modified rolling circle amplification (Wu et al., 2006) and labeled with cyanine 5-dUTP.

Labeled samples were hybridized in triplicate to the GeoChip 2.0 (He et al., 2007). The GeoChip 2.0 contains ~24,000 probes for various functional gene groups.

Arrays were imaged and analyzed using ImaGene software (v.6.1.0, Biodiscovery Inc.)

Signal-to-noise ratios (SNR) was used to determine positive probes.

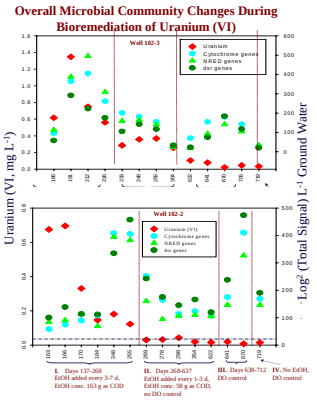
Functional Gene Categories Covered by GeoChip 2.0

Gene Category	Unique probes	Group probes	Total
Carbon cycling	3896	1414	5310
Nitrogen cycling	3708	891	4599
Sulfate reduction	1286	329	1615
Phosphate utilization	89	56	145
Metal reduction and resistance	4039	507	4546
Contaminant degradation	6941	1087	8028

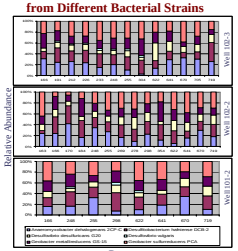
RESULTS

1. Pilot scale system for biostimulation of U(VI) reduction – U(VI) reduction phase

Overall Microbial Community Changes During Bioremediation of Uranium (VI)

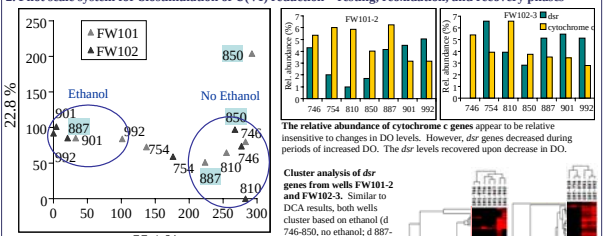


Relative Abundances of Cytochrome C Genes from Different Bacterial Strains



- Based on the cytochrome c genes detected, a population shift in metal reducing bacteria was observed over time.
- The relative abundances of *Anaeromyxobacter* and *Desulfitobacterium* increased as *Geobacter* spp. decreased.
- Changes in wells 102-2 and 101-2 occurred at similar times. The changes in well 102-3 may have occurred earlier so we were unable to detect the first half of the peak.

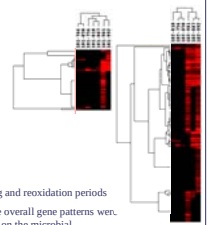
2. Pilot scale system for biostimulation of U(VI) reduction – resting, reoxidation, and recovery phases



Detrended Correspondence Analysis (DCA) results of array data from the two monitoring wells. Samples clustered into two main groups (circled) – one of wells during resting (no ethanol addition) and reoxidation periods during which no ethanol was injected (right) and the other after oxygen control was reestablished and ethanol injections were restarted (left). This suggests that the communities were significantly altered by resumption of ethanol injections.

The relative abundance of cytochrome c genes appear to be relative insensitive to changes in DO levels. However, *dsr* genes decreased during periods of increased DO. The *dsr* levels recovered upon decrease in DO.

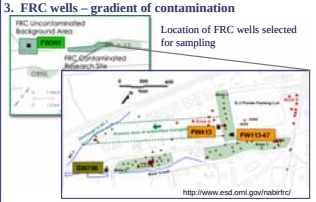
Cluster analysis of *dsr* genes from wells FW101-2 and FW102-3. Similar to DCA results, both wells cluster based on ethanol (d 746-850, no ethanol; d 887-992, ethanol). Clustering also indicates that well FW101-2 was more affected by the increased DO than FW102-3.



A population shift occurred after ethanol was reintroduced into the system after the resting and reoxidation periods

While the introduction of DO affected the relative abundance of some gene categories, the overall gene patterns wer. similar to those seen during the resting period, suggesting DO did not have a great impact on the microbial community

3. FRC wells – gradient of contamination



Geochemistry of selected wells

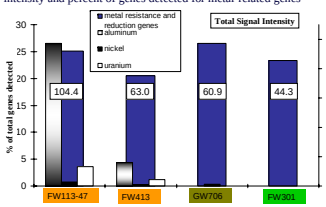
Sample	pH	NO_3^- (mg/L)	SO_4^- (mg/L)	U (mg/L)
FW113-47	3.44	5811	2660	66.96
FW413	3.75	706	1193	21.45
GW706	7.29	79.8	23.9	0.06
FW301	5.46	0.47	4.92	0

Percent overlap of functional gene diversity

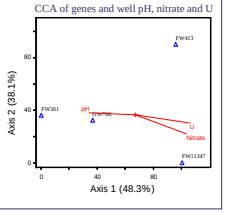
Sample	FW113-47	FW413	GW706	FW301
FW113-47	*32.16%**	53.22%	49.71%	29.82%
FW413		*30.99%**	47.37%	32.75%
GW706			*15.79%**	58.48%
FW301				*35.09%**

** % of genes unique to that well*

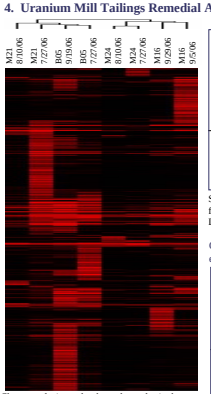
Relationship between metal concentrations and the total signal intensity and percent of genes detected for metal-related genes



CCA of genes and well pH, nitrate and U

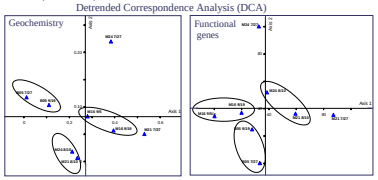


4. Uranium Mill Tailings Remedial Action (UMTRA) site, Rifle, CO



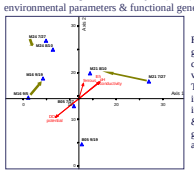
Cluster analysis results showed samples in the same locations grouped together, regardless of geochemistry.

Detrended Correspondence Analysis (DCA)



Samples under iron-reducing (M16) and sulfate-reducing (M24 & M21) conditions and those from the background site (B05) grouped into separate clusters. Community and environmental DCA results were similar.

Canonical Correspondence Analysis (CCA) of environmental parameters & functional genes



Ferrous iron was the most significant geochemical variable associated with community structure. The CCA model with six variables is relatively strong (p=0.1051). The y-axis is defined almost solely by ferrous iron. With acetate injection, samples under iron-reducing (M16) and sulfate-reducing (M21 & M24) conditions appeared to be strongly governed by ferrous iron (as shown with green arrow).

5. Lake DePue sediment microbial communities

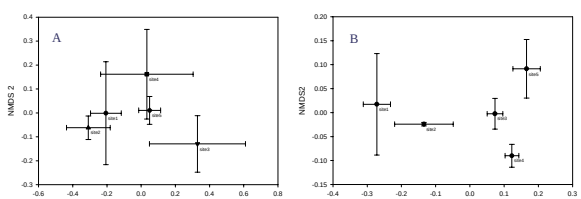


Fig. 1. NMDS plot of microbial communities (A) and all environmental variables (B) with all available functional genes. Error bars represent 1 standard error.

Lake DePue has been contaminated for last ~80 years from a nearby zinc smelting facility and a small man-made creek running from the facility to the lake near sites 1 & 2.

Partial Mantel test with geographic distance held revealed a close relationship between the entire microbial community and pore water metal concentrations with sediment characteristic variables (r_M = 0.264, p = 0.063). Overall correlations with all environmental variables were less significant (r_M = 0.218, p = 0.106).

Microbial communities were not spatially autocorrelated, while environmental variables were significantly autocorrelated (p = 0.014), as indicated by the NMDS plot (B) which reflect actual geographic configuration of sampling locations.

SUMMARY

The GeoChip 2.0 is the first comprehensive microarray available for studying the functional and biogeochemical cycling potential of microbial communities.

GeoChip 2.0 is able to provide a large amount of data regarding the functional microbial community present at sites of interest, which can then be used in a variety of analytical methods to gain a greater understanding of the community itself and how environmental factors affect the community composition.

The studies presented here demonstrate the ability of the GeoChip to monitor the dynamic functional and structural changes in microbial communities. The GeoChip is able to detect differences between microbial communities which can the be correlated with spatial, temporal, and environmental factors.

ACKNOWLEDGEMENT

ESPP2 (MDCASE) is part of the Virtual Institute for Microbial Stress and Survival (VIMSS) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

Contact information: joy.vannostrand@ou.edu; jzhou@ou.edu